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BY:

G.A. Luoma
Detection and Identification Section Head
Defence Research Establishment Suffield
Box 4000, Medicine Hat, Alberta
T1A 8K6

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DETECTION AND IDENTIFICATION SECTION PUBLICATION RECORD 1995/96

BY
GREG LUOMA
DETECTION AND IDENTIFICATION SECTION HEAD

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Abstract

This document represents a collation of the publications for the Detection and Identification Section at the Defence Research Establishment Suffield for the calendar year of 1995 and January to May 1996. The publications have been divided into three types: (1) DRES publications; (2) Contractors' Reports, and; (3) External Publications.

The DRES Publications represent all of the in-house publications based on research and development in support of CF requirements. Executive summaries which emphasize the defence relevance of the work are included in this section, and full reports are available from DRES. The Contractors' Reports represent all of the final reports received for work conducted under contract to the Section. Although executive summaries are not included, the full reports are available from DRES. The External Publications section contains a bibliography of all of the articles published or submitted to scientific journals for publications. These articles are available from either DRES or any library which carries the appropriate journal.

The intent of this publication is to provide a brief review of the achievements of the Detection and Identification Section at DRES over the last 18 months in a format that is meant for both technical and non-technical audiences. If there are any questions about the program or any of the publications please contact the undersigned.

Dr. Greg A. Luoma
Head, Detection and Identification Section
DRES
PO Box 4000
Medicine Hat, AB T1A 8K6
tel: (403) 544-4672
fax: (403) 544-3388
e-mail: greg.luoma@dres.dnd.ca
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A. DRES Reports

Yee, E., "An Assessment of the Effects of Concentration Fluctuations on the Penetration of Toxic Vapours Through A Carbon Bed", **Suffield Memorandum No. 1457**, 1995.

Introduction

Current models for chemical and biological warfare (CBW) hazard predictions and NBC survivability modelling are based on using standard atmospheric dispersion methods for estimating the mean concentration of a toxic gas or aerosol at a given downwind location. This mean concentration represents an integrated average over an ensemble of many identical releases under nominally the same meteorological conditions, and is frequently used with exposure time (dosage level) for risk assessment and for determination of probable challenge levels for assessing the protection afforded by CBW protective equipment (e.g., CBW suits, gas masks, etc.). However, this simple approach of only describing the downwind cloud dilution process in a time-averaged sense (viz., the mean concentration over a given time) completely ignores the fact that natural variability of toxic gas or aerosol dispersion in the turbulent atmosphere will produce a range of mean and fluctuating concentrations at any fixed location downwind of a CBW release. In consequence, the mean concentration that is used for hazard prediction and assessment may not be very representative of the actual or instantaneous concentrations that exist at any particular downwind location. In particular, the mean concentration is neither the median concentration nor the most probable concentration, nor does it provide any information on the worst case extreme concentrations that may occur. Consequently, the reliability of CBW hazard predictions based on mean concentrations may be severely limited.

Naturally induced fluctuations in atmospheric concentrations of CBW agents are a ubiquitous feature of all contaminants dispersing in the atmospheric boundary layer. The random fluctuations in concentration resulting from the atmospheric turbulence can be clearly seen with a high-resolution concentration sensor. In consequence, the practical importance of concentration fluctuations in CBW hazard predictions must be stressed, the mean concentration alone is insufficient for hazard analysis, as is clear in a number of military assessment problems. For example, it is now known that concentration fluctuations can yield a significant enhancement in the degree of poisoning and percentage mortality resulting from exposure to CBW agents in which the interaction of concentration and exposure time is nonlinear (e.g., toxic materials that do not abide by Haber's rule). Still another example of military significance arises in the release of a potentially flammable cloud of gas (e.g., fuel-air explosive) on the battlefield environment in which ignition and explosion of the cloud is known to depend critically upon the instantaneous concentration lying between the lower and upper flammability limits, irrespective of the mean concentration.

In this paper, we examine the importance of concentration fluctuations in the determination of vapour penetration through an adsorbent (activated) carbon bed. To address this problem, a simple model for the removal of a gaseous adsorbate from a flowing air stream by an adsorbent bed (e.g., removal of a toxic gas by charcoal) has been used to assess the role of concentration fluctuations on the vapour penetration of an adsorbent bed. The model is based on a mass balance continuity relationship in the adsorbent bed, with the implicit assumption that the local rate of removal of gas in the bed is an irreversible process (viz., no desorption occurred) with surface adsorption as the rate controlling step. The model shows that the exit concentration from the bed at any time depends on the inlet concentration at the retarded time (related to the residence time of the vapour in the bed), and on the partial dosage provided by the inlet concentration. The latter suggests that the time to break or penetrate an absorbent bed and the degree of penetration through the bed must depend on the inlet concentration pattern (viz., the importance of concentration fluctuations about the mean at the bed entrance cannot be ignored or undervalued).

Results

We have substantiated this effect by using some concentration fluctuation data obtained as part of a cooperative Concentration Fluctuation Experiments (CONFLUX) project involving three defence research establishments in the United States, Great Britain, and Canada. To this purpose, we compared the vapour penetration through a carbon bed for two different exposures with equal dosage; namely, a steady inlet concentration and a time-varying (fluctuating) inlet concentration. The results of the simulation showed that there is a significant effect of concentration fluctuations on the vapour penetration through the carbon bed. This example indicated that for the two exposures, the fluctuating concentration challenge produced a significant enhancement in the penetration (about 25-fold) and a reduction in the breakthrough time (about 50%) compared with the steady concentration challenge. Hence, use of the mean concentration alone in assessment of protection afforded by respiratory devices without any account of inherent fluctuations may lead to an underestimation of the protection factor provided by such equipment (at least for the simple local rate of removal mechanism assumed for the present model).

However, it should be emphasized that the model used here for the investigation of the vapour penetration through a carbon bed is highly idealized. In particular, it was assumed that the vapour in the bed was adsorbed irreversibly (i.e., no desorption) with the local rate of removal taken to be first-order with respect to the vapour concentration and the concentration of unoccupied adsorption sites. This restrictive assumption was imposed in order to facilitate a complete analytical mathematical solution for this simplified removal process in which the effect of a fluctuating inlet concentration is explicitly exhibited. However, to simulate the performance of a filter in a more realistic situation, the model used here with its fluctuating concentration inlet condition will have

to be extended to incorporate a more realistic mechanism for the local rate of vapour removal in the bed (e.g., nonlinear mass rate of transfer between the vapour and adsorbent surface; effects of the heat of adsorption of the vapour in the bed; different types of isotherm behaviour such as nonlinear and irreversible, Langmuir, Fruendlich, etc.; inclusion of both adsorption and desorption in the removal process; influence of relative humidity on the penetration, etc.). It should be emphasized that the latter effects need to be considered in relation to the effects of a fluctuating inlet concentration (such as that from a cloud dispersing in the atmosphere) if one is to predict or simulate the performance of a charcoal filter under "real" conditions.

Significance of Results

The importance of concentration fluctuations in military hazard predictions highlights the need to develop models that incorporate greater physical reality (e.g., incorporation of the statistical characteristics of a fluctuating concentration) than is available in current dispersion models. These concerns have placed an additional premium on the value of reliable concentration fluctuation data obtained from field tests, and on the use of this data to guide and develop statistical or probabilistic models for hazard assessment. The work reported here is but one further example of the inadequacy of using the mean concentration alone in a model, without any account of the fluctuations (viz., of using a purely deterministic model in the prediction of a stochastic or random process such as gas dispersion in the atmosphere). The point of this comment is not that modelling the mean concentration is not worthwhile, but that efforts that totally ignore or undervalue the importance of concentration fluctuations about the mean may be misguided and/or unrealistic because they cannot yield "accurate" predictions except in a very cosmetic or superficial sense.

Future Goals

In view of the statistical nature of a fluctuating concentration, and the fact that the latter constitutes the inlet challenge to charcoal filters used in real military situations, it is recommended that probabilistic models be developed for prediction of the effectiveness of charcoal filters for protection from CW agents. The latter models will be more realistic in the sense that they explicitly recognize and account for the stochastic nature of the turbulent dispersion process in the atmosphere that produces the fluctuating concentrations that challenge all CBW protective equipment in real situations.

Camille A. Boulet and Carol Townsley, "Capillary Zone Electrophoresis Analysis and Detection of Mid-Spectrum Biological Warfare Agents", **Suffield Memorandum No. 1463**, April 1995.

Introduction

DRE Suffield has initiated a research program to develop methods and equipment for field detection and laboratory identification of mid-spectrum biological warfare agents, molecules of biological origin such as proteins, peptides and toxins. These mid-spectrum agents are often difficult to analyse and often require individually developed bio- or immunoassay methods for detection and identification. A chromatographic-based analytical system which could separate and detect all of the target compounds would represent an important advance in detection and identification capability. Additional or new mid-spectrum agents can be more easily accommodated in a chromatographic analytical system as these systems use a generic principle for detection rather than custom prepared immunoassays.

Capillary electrophoresis (CE) is an important, emerging technique for the separation and quantisation of peptides and proteins providing separation efficiencies up to two orders of magnitude greater than high performance liquid chromatography (HPLC). It can analyse a broad range of compounds, has a simple instrument design which can be fully automated, and has very low volume requirements for both sample and run buffers which would substantially reduce the maintenance and logistics burden for fielding a CE based instrument for biological detection. CE also uses a novel principle for liquid handling, electro osmotic flow, which requires no moving parts to transfer reagents that could be adapted to other analytical devices.

Results

In this study, a highly efficient and reproducible capillary zone electrophoresis method was developed to separate and identify a series of nine peptides of defence interest: bradykinin, bradykinin fragment 1-5, substance P,[arg^8]-vasopressin, luteinizing hormone releasing hormone, bombesin, leucine enkephalin, methionine enkephalin, and oxytocin. Several of these peptides have been identified as potential mid-spectrum agents. Three strategies, which could be used in a fully automated field detection and identification system, were demonstrated for the identification of unknown peptides: comparison of migration times, comparison of electrophoretic mobilities, and co-injection of multiple reference standards.

Significance of Results

These experiments demonstrate that a separation based analytical method such as capillary electrophoresis could form the basis of a generic detection system for mid-

spectrum protein and peptide toxins.

Future Goals

The use of capillary electrophoresis for the field identification of mid-spectrum agents will be pursued. A field portable system is being developed under contract and will be evaluated at joint field trials with the US and UK at Dugway, Utah. If successful, capillary electrophoresis will be considered for inclusion in the advanced development model of the Canadian Integrated Bio-chemical Agent Detection System (CIBADS).

Bader, D.E., "Construction of a Recombinant Viral Vector Containing Part of the Nucleocapsid Protein Gene of Newcastle Disease Virus", **DRES Suffield Memorandum No. 1464**, 1995.

Introduction

The Canadian Forces have a basic requirement to be able to function within a biological warfare (BW) environment. Early warning detection and identification of biological agents is the first line of defence against an attack. A key technology that is being studied at Defence Research Establishment Suffield (DRES) for identification of biological agents is gene probe technology. Gene probes are pieces of genetic material that are selected to match unique target nucleic acid sequences of suspected biological agents through the process of complementary molecular recognition. Gene probes play a key role in identification of biological agents because of the capability to identify genetic material regardless of its source, thereby affording identification of conventional agents such as viruses, bacteria, rickettsia or fungi, as well as potential threats, such as harmless organisms containing cloned threat agent genes or micro-encapsulated infectious nucleic acid.

Gene probe identification of very small quantities of target nucleic acid is made possible by using molecular biological techniques which increase the number of copies of a target sequence (amplification). One of these techniques, called polymerase chain reaction or PCR, uses short DNA sequences called primers, that bind to the DNA target sequence and set the boundaries of the region of the DNA to be amplified (when RNA is the target for PCR amplification, the RNA must be converted to DNA prior to PCR using an enzyme called reverse transcriptase). The PCR process generates multiple copies of this DNA sequence with a defined size or length. Size information can be used as a means of identification, if the primers complement unique or agent-specific sites on the target sequence. Further evidence that the correct DNA fragment has been amplified, can be obtained by probing the amplified product with a gene probe that is designed to bind to a target sequence within the amplified fragment.

We have designed PCR primers and gene probes for Newcastle disease virus (NDV). This has been done for two reasons. First, NDV has been developed as a BW viral simulant for use in field experiments at DRES. Second, many viruses that could be potential BW threats are RNA viruses and since the genomic nucleic acid component of NDV is RNA, NDV serves as a useful model for developing methodologies for the identification of RNA viruses. The PCR primers and gene probes thus far used, have been designed for the major nucleocapsid protein gene. This gene was selected as a target because it is part of the nucleocapsid structure unit of the virus which has been suggested to be a necessary requirement for negative-stranded RNA viruses. Because of its importance to the virus, it is likely that this structure is highly conserved among the negative-stranded RNA viruses and therefore this gene should be a good target with

which to design negative-stranded RNA viral gene probes. Part of the major nucleocapsid gene sequence has been generated by RT-PCR and used in DRES studies. This report describes the process of cloning this gene sequence into a viral DNA vector molecule to construct a recombinant DNA molecule which is easily stored within and recovered from a bacterial host cell line, thereby allowing us the capability to maintain and recover this sequence in unlimited quantities for future studies.

Results

The nucleocapsid protein gene fragment was successfully cloned into the viral vector M13mp18 RF to produce a recombinant DNA molecule. The recombinant DNA was shown to contain the cloned fragment based on four lines of evidence. First, bacterial cells transformed with the recombinant DNA, formed clear plaques in the presence of indicator dyes while bacterial cells transformed with vector DNA, produced blue plaques. Second, recombinant DNA that was isolated from clear plaques and was treated with the same restriction enzymes that were used in the cloning process, generated a fragment of similar size to the cloned gene fragment. Third, a positive signal was generated for the recombinant DNA but not for the vector DNA, following hybridization with a gene probe designed to bind to the cloned gene fragment. Finally, PCR amplification of the recombinant DNA using a set of nested PCR primers (primers located within the original primer pair) generated an amplified product (amplicon) of the expected size following agarose gel electrophoresis. The nested amplicon was probed with the NDV gene probe and found to generate a positive signal.

Significance of the Results

The cloned nucleocapsid gene fragment will allow DRES to maintain an unlimited source of this sequence for future studies. This will yield a source of specific gene probes for NDV to be used in the development and evaluation of field portable biological agent identification systems.

Future Goals

The recombinant DNA will be maintained inside *E. coli* host cells and isolated for future studies into the development of field portable biological agent detection systems.

J.R. Hancock, P.A. D'Agostino and L.R. Provost, "Identification of Ile-Ser-Bradykinin in Aqueous Samples by Liquid Chromatography with Ultraviolet and Electrospray Mass Spectrometric Detection", **Suffield Memorandum No. 1465**, 1995.

Introduction

The Canadian Forces (CF) may be called on to perform peacekeeping or peacemaking operations in regions of the world where there is a significant threat of chemical/biological warfare agent use. To operate effectively in these theatres the CF must be able to identify the exact nature of the chemical/biological agent(s). Liquid chromatography-mass spectrometry (LC-MS), is a powerful analytical technique for the resolution of compounds in complex sample matrices. DRE Suffield is currently investigating this instrumental technique in fulfilment of CF chemical warfare agent identification requirements.

Results

A bioactive peptide, He-Ser-Bradykinin, was selected to evaluate sample handling and analysis methods for the detection and identification of mid-spectrum agents in aqueous samples. Ile-Ser-Bradykinin was spiked at the 0.05 mg level into distilled water, slough water and water produced by the Large Volume Air Sampler (LVAS), proposed for the Canadian Integrated Biological Agents Detection System (CIBADS). Spiked samples and standards were filtered and analysed at several intervals over a 28 day period to simulate collection and subsequent analysis of aqueous field samples for mid-spectrum agents. In order to recover Ile-Ser-Bradykinin from these samples, it was necessary to add 0.1% trifluoroacetic acid to the sample prior to filtration through a Millipore Millex HV4 filter. Ile-Ser-Bradykinin was readily detected and identified by liquid chromatography (LC) with ultraviolet (UV) and electrospray mass spectrometric (ESI-MS) detection in all the water samples during the first day, but had degraded to a significant extent within eight days in samples kept at room temperature. When stored at -18°C Ile-Ser-Bradykinin standards showed no signs of degradation, even after 28 days. A sample detection limit of 3 ng (2.5 pmole) was estimated for Ile-Ser-Bradykinin under LC-ESI-MS conditions.

Significance of Results

The CF, during active duty in regions of the world where there is a significant threat of chemical/biological warfare agent use, could encounter use of irritants in a warfare role. Intelligence gathering, through the collection of contaminated samples, and subsequent analysis of the samples would lead to the identification of the compound used. The Canadian chemical/biological identification capability resides at Defence Research Establishment Suffield, where analytical specialists confirm and identify chemical warfare agents in suspect samples for the CF. The results of such analyses would

contribute to the development of strategic and political positions regarding future Canadian military operations and would facilitate the dissemination of technical advice to in-theatre field commanders and medical personnel.

Future Goals

The chemical/biological threat spectrum includes classical chemical and biological warfare agents and toxins of biological origin in the "mid-spectrum" between the classical warfare agents and biological warfare agents. The identification research effort will be focused on the detection and identification of toxins of biological origin. Use of these warfare agents could easily go unconfirmed, as analytical methods have not yet been developed for their identification. DRE Suffield is now actively addressing this deficiency through the application and development of sample handling techniques and MS methods for these toxins.

Bader, D.E. and Lewis, J. "Development of a Microplate Gene Probe Assay for Newcastle Disease Virus", **DRES Suffield Memorandum No. 1470**, 1995.

Introduction

The Canadian Forces (CF) have a basic requirement to be able to function within a biological warfare (BW) environment. Early warning detection-identification is the first line of defence for such a scenario. The Defence Research Establishment Suffield has been tasked to develop a field-based BW detection-identification system. Because of the diversity in the nature of the BW agents that could be used against the CF (bacteria, viruses, microbiological toxins, human effector molecules), it is currently impossible to apply a single technology that is capable of identifying all potential BW threat agents. Therefore, several technologies are being evaluated including mass spectrometry, capillary electrophoresis, antibody-based technologies, and gene-based technologies.

Gene-based technologies address not only the classical biological agents (e.g., virus, bacteria, rickettsia, fungi), but also newly emerging threat agents that could arise as a consequence of advances in molecular biology and biotechnology, for example, innocuous organisms containing cloned biological toxin genes or micro-encapsulated infectious nucleic acid. We are currently evaluating gene probes (short pieces of genetic material that bind to a complementary nucleic acid sequences in a sequence specific manner) as tools for biological agent identification. We have developed a membrane-based gene probe assay for the RNA virus simulant Newcastle disease virus (NDV). Conventional gene probe assays are performed on nylon membranes because of their ability to bind large amounts of DNA and their ability to be stripped for re-probing. While this format is the method of choice for laboratory-based identification strategies, a microtiter plate format may be a better choice for field-based identification due to a greater capability for automation and machine-readable quantitation. The identification technology used in the Mobile Agent Identification Unit (MAGIDU) that was sent to the Gulf during Operation Desert Storm by DRES for the identification of biological agents, was an enzyme-linked immunosorbent assay that utilized the microtiter plate format. In order to incorporate gene probe identification capability into the MAGIDU and to take advantage of enhancements in ease of performance and handling, speed, automation, and machine-readable quantitation over membrane-based assay formats, we have developed a gene probe assay using a microtiter plate format. This study describes the development of a microtiter plate gene probe assay for the detection and identification of Newcastle disease virus.

Results

Initial assay parameters (selected based on previously published methods) allowed for the detection of target NDV DNA over a range of $8x10^7$ to $7x10^9$ molecules under low stringency conditions (Tm-32°C). Treatment of the plates with UV light improved the

lower limit of detection compared to non-UV treated plates within the same trial, and as such, was adopted. Optimum probe and detector antibody concentrations were selected based on signal-to-background ratios. Assays performed under these optimized conditions resulted in a slight improvement in the lower detection limit which was demonstrated to be $2x10^7$ molecules of NDV673 DNA under low stringency conditions (Tm-32°C). For comparison purposes, our membrane-based, colorimetric assay for the same probe/target system had a lower detection limit of 10^5-10^6 molecules under more stringent conditions (Tm-1.4°C) suggesting that the microtiter plate assay is not as sensitive.

Evaluation of four other plate types indicated only one (Immulon 1) that showed any advantage over the Maxisorp plate used in the optimization studies. The Immulon 1 plate generated similar lower detection limit values but generated higher signals and a greater rate of change of signal over the target range tested, indicating greater sensitivity.

Significance of Results

The data suggests that the lower detection limit for microplate gene probe assays is not as low as that of membrane-based gene probe assays. In addition, significant reductions in assay time relative to membrane-based assays were not realized. However, many of the other characteristics which make microtitre plates a better choice for fieldable identification systems were realized. For example, they were much easier to work with than membranes, being less cumbersome and easier to handle. Many of the steps were performed by automated equipment (plate washers and readers), an advantage of great importance as automation lends itself to better accuracy, fewer time-consuming steps, less experimenter bias and less manual labour. Machine-readable quantitation using the automated colorimeter was rapid and easily performed. Machine-readable quantitation for membrane-based non-radioactive assays on the other hand, would require the use of densitometry which is a more time-consuming and tedious method, requiring more expensive equipment.

Future Goals

In an effort to improve analysis speed even further, DRES has begun studies to couple gene probe technology with biosensor technology. Consequently, in-house studies have been initiated to detect nucleic acid target analyte using solution phase hybridization in conjunction with shorter gene probes (oligonucleotides), followed by detection of the probe/target complex using a light addressable potentiometric sensor.

Bader, D.E., Fisher, G.R. and Lee, W.E., "Gene Probe Assay of Viral Nucleic Acid Using a Silicon Biosensor", **DRES Suffield Memorandum No. 1471**, 1996.

Introduction

Gene probe assays are an increasingly important technology in detection and identification of biological agents, as well as medical diagnostics. Gene probe assays use discrete sequences of nucleic acid (DNA or RNA), that are complementary to distinct regions of the genetic material being analysed. Since nucleic acid is a component of all living material, probes can be devised and used against nucleic acid from essentially all sources including viruses, bacteria, plants or animals. In addition, gene probes can be used to identify genetic sequences that have been transferred naturally or by human design into foreign vehicles, for example, toxin genes cloned into innocuous organisms or infectious nucleic acid contained within man-made microcapsules. Gene probe assays are conventionally performed as mixed-phase assays whereby the target DNA is bound to the solid phase and the probe hybridizes to the target in solution phase. Mixed-phase assays, while being very sensitive, tend to be slow because extra time is required to bind target to the solid phase and block the solid phase following target binding. In addition, mixed-phase reactions are not as kinetically favoured as solution-phase reactions with reaction rates being up to 10 times slower. These types of assays are also more technically demanding and cumbersome. Any improvements in reducing assay time and complexity, without compromising sensitivity, would be beneficial to the utility of gene probe assays for identification purposes. This preliminary study describes the development of a solution-phase assay format using a commercially available biosensor-based detection apparatus (LAPS) and compares it to a conventional mixed-phase approach in terms of assay sensitivity, speed and ease of use. A DNA fragment of the nucleocapsid protein gene of Newcastle disease virus was used as the target DNA.

Results

The biosensor-based assay was characterized as a function of temperature, hybridization time, and probe concentration. The lower limit of detection was found to be about 0.3 fmol of target DNA (1.8×10^8 molecules) under low stringency conditions (Tm-22°C). Total assay time including hybridization and potentiometric sensing was about 45-60 min.

Significance of Results

Comparative analysis of these results with those obtained for a mixed-phase membrane-based assay using the same probe/target system suggests that the biosensor assay generates equivalent detection limits but in much less time. In addition, the biosensor-based assay is much less technically demanding.

The LAP sensor apparatus has been demonstrated in previous DRES studies to be effective as an analytical system to carry out immunoassays for protein (toxins), virus, bacteria, and anti-cholinesterase organophosphorous compounds. The immuno-based LAPS system was recently adopted as one of two identification technologies for integration into the Canadian Integrated Biological Agent Detection System (CIBADS) which performed well during recent field trials undertaken at Dugway Proving Grounds (USA) in September 1995. This study demonstrates the expanded capability of the LAP sensor to carry out rapid, sensitive gene probe assays with the potential for integration into the CIBADS.

Future Goals

More studies are required to determine whether the gene probe-based LAPS assay performs as well as membrane-based assays under more stringent hybridization conditions. Higher stringencies may be required in order to improve specificity of the probe/target interaction.

There is good potential for the gene probe-based LAPS assay to be integrated into CIBADS and tested under field trial conditions, however, there are other commercially available biosensor systems that could offer significant advantages over the LAPS. For example, surface plasmon resonance or SPR, does not require secondary detection of probe/target analyte, thereby reducing detection time as well as reducing reagents and consumables. Gene probes will continue to play an important role in identification strategies being developed for the CF.

Bader, D.E. and Gray, D., "Evaluation of a Sandwich Gene Probe Assay for Newcastle Disease Virus", **DRES Suffield Memorandum No. 1474**, 1996.

Introduction

Gene probe assays are an increasingly important technology in detection and identification of biological agents, as well as medical diagnostics. Gene probe assays use discrete sequences of nucleic acid (DNA or RNA), that are complementary to distinct regions of the genetic material being analysed. Since nucleic acid is a component of all living material, probes can be devised and used against nucleic acid from essentially all sources including viruses, bacteria, plants or animals. In addition, gene probes can be used to identify genetic sequences that have been transferred naturally or by human design into foreign vehicles, for example, toxin genes cloned into innocuous organisms or infectious nucleic acid contained within man-made microcapsules. Gene probe assays are conventionally performed as mixed-phase assays whereby the target DNA is bound to the solid phase and the probe hybridizes to the target in solution phase. We have previously developed a mixed-phase gene probe assay for Newcastle disease virus using nylon membranes as the solid phase. The gene probe used in each of these assays was a 673 bp DNA fragment of the major nucleocapsid protein gene of Newcastle disease virus (NDV). The 673 bp sequence was labelled with digoxigenin, a non-radioactive label, and used as a probe against unlabelled 673 bp DNA. This assay format is termed a direct assay since the bound target DNA is hybridized directly with a gene probe.

An alternative assay format, called a sandwich assay, has been described in the literature which uses two probe sequences. One probe sequence recognizes and binds to the target analyte and the other probe sequence recognizes and binds to the first probe sequence. The implication of the sandwich assay format is that it may allow for enhanced assay sensitivity through the formation of networks (multi-labelled complexes) brought about through the overlapping of complementary regions of the two gene probes. We were interested in determining whether this sandwich assay format would result in greater sensitivities than the direct gene probe assay. This report describes such a study.

Results

The sandwich assay resulted in detection limits similar to those demonstrated for the direct assay (10^5 molecules of purified target DNA) when molar probe concentrations between the two assays were around 20 pM. When molar probe concentrations in the sandwich assay were increased beyond this, sensitivity decreased and background problems due to non-specific binding became evident.

Significance of Results

The sandwich assay did not offer any advantage over the direct assay with respect to sensitivity or ease of use. These results validate the use of the direct assay format as the method of choice for probe assays being developed at DRES.

Future Goals

The goal of this study and recent gene probe studies at DRES has been to develop a working knowledge of the utility of gene probes as tools for the identification of biological agents, with emphasis on assay design, detection limits and ease of use using the viral model Newcastle disease virus. We have gained experience in gene probe design using NDV as a model organism and will use this knowledge to design and procure gene probe sequences for biological agents of concern to the CF. We have and will continue to evaluate gene probe related technologies that improve upon our current capabilities with regards to sensitivity, specificity, simplicity, rapidity, automation and miniaturization. Other areas of study include developing simple, rapid sample preparation techniques together with evaluation of candidate gene probe methods for the analysis of test analytes in environmental samples such as air, water and soil.

Yee, E., "A Non-Linear Dose-Response Model With an Application to the Reconstruction of the Human Mortality Response Surface From Acute Inhalation Toxicity With Sarin", **Suffield Memorandum No. 1476**, 1996.

Introduction

There are, quite rightly, growing concerns world-wide about the dangers, both actual and potential, of the use of chemical warfare (CW) and biological warfare (BW) agents. Such concerns are causing governments and their defence agencies to develop CBW agent hazard assessment strategies and paradigms that can be used for the formulation of operational and design procedures for regulating and managing the consequences of the hazard. As well, there is an urgency to provide answers to a number of political and military concerns regarding the use of CBW agents. Frequently, the formulation of hazard assessment schemes for CBW agents requires mathematical modeling. This modeling is based principally on turbulent diffusion calculations that are used to generate downwind hazard distance data which, subsequently, need to be translated into specific levels of harm to exposed military personnel.

Assessing the likely damage from exposure to CBW agents involves three principal modeling components, each of which introduces an uncertainty in the hazard assessment process. The first component concerns a knowledge of the characteristics of the source. This typically includes input information such as the kind and amount of material released, the duration of the release, the source location and geometry, etc. Naturally, there are uncertainties in estimating each of these source characteristics. The second component involves the transport and diffusion of the material (contaminant) through the turbulent atmosphere. On a fundamental level, this involves modeling the advection by the mean wind and dilution by the atmospheric turbulence, but because of the diversity of CBW agents, this stage may frequently involve other physical processes as well (e.g., for CW agents, this stage may involve two-phase phenomena with significant thermodynamic effects, frequently leading to negative buoyancy [or dense gas] effects; for BW agents, a number of non-conservative processes may be involved such as gravitational settling, wet and dry deposition, biological activity agent decay, etc.). The third component is an estimation of the effects (e.g., toxic, infective, etc.) on the exposed military personnel as a function of distance from the CBW agent release.

In this study, we are concerned principally with the estimation of toxicological impact of a CBW agent release on the exposed military personnel in the surrounding area. In particular, we address the uncertainties arising from the determination of the human toxic response to CBW agents from the adaptation of animal toxicity data to human mortality. In a military hazard assessment which is taken to the point of determining the toxicological risk on the soldier, it is necessary to estimate injury (or lethality) in the area around the CBW agent source. In this paper, it is argued that a non-linear dose-

response model can be used to achieve this objective.

Results

In recent years, it has been found that the injury produced by many common industrial gases (e.g., chlorine, ammonia, etc.) can be assessed in terms of a non-linear toxic load in which the exacerbating effect of a concentration peak is modeled by the simple expedient of weighting the concentration by a toxic load exponent n. The exponent n can vary considerably depending on the particular toxic gas. Indeed, n can be greater than one, implying that concentration rather than exposure time is the dominant factor in determination of the toxic response. In general, the values of n have been found to lie in the range from about I to 4 for a wide variety of industrial toxic gases. Although the industrial gases appear to have been investigated extensively and shown to violate generally the concept that the product of concentration and exposure time correlates well with the mortality response (e.g., Haber's rule), the same information does not appear to be available generally for the CBW agents (e.g., pulmonary, nerve, psychotoxic, and toxin warfare agents).

The use of a non-linear toxic load in conjunction with the probit method for the characterization of the time-concentration-mortality relationship of a toxic gas is described. The basic principles underlying the extrapolation of the time-concentration-mortality relationships from animal to humans are examined. These methods are illustrated with reference to the re-evaluation of the raw data from a previously published study of a large-scale experiment on the acute inhalation toxicity of sarin (GB). For the four animal species (e.g., rats, mice, guinea pigs, and pigeons) used in this study, the parameters in the probit relationship (including the toxic load exponent) have been derived using a maximum likelihood method based on the binomial distribution. From these results, a human acute inhalation toxicity model for G13 intoxication is developed. This model can then be used to provide an estimate for the LC_{01}, LC_{05}, and LC_{50} values for humans as a function of the exposure time.

Significance of Results

The non-linear dose-response models used in this paper (as well as the adaptation of these models of animal toxicity to human mortality) have a direct application in quantitative military hazard assessment. Specifically, the models permit an estimation of the probability of lethality from exposure to CBW agents given any concentration and exposure time. In particular, these models define a mortality response surface for a CBW agent that can be used to determine all combinations of concentration and exposure time (within the limits determined by the available toxicological data) that could give rise to a specified level of toxicity (SLOT). Furthermore, the non-linear dose-response models can be used in conjunction with atmospheric dispersion models to estimate the degree of injury or damage to exposed military personnel around a CW or

BW agent release.

A previous report entitled "A Rational Basis for Accounting for the Impact of Concentration Fluctuations on Toxicological Assessment and Estimation of Injury Resulting From the Release of Chemical and Biological Warfare Agents" (SR-634, Defence Research Establishment Suffield) described a rational, consistent, and simple methodology for accomplishing this task. The latter study emphasizes the enhancement of the toxicological risk arising from a fluctuating concentration, and how a neglect of concentration fluctuations about the mean can lead to a serious underestimation of the level of damage. A more detailed technical description of the correct method for combining the non-linear toxic load with a fluctuating plume concentration for risk analysis, and the implications of these results for determining the fraction of an exposed military population that will experience a SLOT from a random toxic load are discussed in detail in the paper entitled "Statistical Characteristics of the Non-linear Toxic Load Derived for Fluctuating Concentrations in a Plume Dispersing in the Atmospheric Surface Layer" by Yee and Chan (in preparation). The results presented in this previous work, when used in conjunction with the non-linear dose-response models described here, permit a completely probabilistic or statistical assessment of damage (e.g., incapacitation, lethality, etc.) likely to be caused by the release of CBW agents into the atmosphere.

Future Work

Before the latter methodology can be used, there is a need to reconstruct the concentration-time mortality response surfaces for the CW and BW agents of interest using the techniques described in this study. In consequence, it is recommended that all acute inhalation toxicity data from animal experiments conducted in the United States, United Kingdom, and Canada to measure the toxicity of the various CBW agents of interest be identified and assembled. The acute inhalation toxicity data of interest here refer primarily to those studies for which both concentration and exposure time have been varied in the laboratory experiments. Furthermore, it is recommended that the raw data from these studies be re-evaluated in terms of the non-linear dose-response model, and that the results of this evaluation be extrapolated from the animal probits to human mortality. In this form, the results would be suitable for use in a quantitative military hazard assessment scheme.

Yee, E., "A Rational Basis for Accounting for the Impact of Concentration Fluctuations on Toxicological Assessment and Estimation of Injury Resulting From the Release of Chemical and Biological Warfare Agents", **Suffield Report No. 634**, 1996.

Introduction

The existing methodology for military hazard assessment is based on using standard atmospheric dispersion methods (e.g., Gaussian plume or puff models) for estimating the mean concentration of a chemical or biological warfare (CBW) agent, and coupling this information with the concept of a linear dosage for determination of the toxic effect on the exposed population. Although the severe limitations of this method for hazard assessment has been understood for over a decade, it is still nevertheless used to estimate the adverse effects that would be felt by an exposed group of people. The widespread use of the current hazard analysis methodology despite its serious limitations derives from a phenomenon that has been termed the availability error. The latter is nothing more than a strong disposition to make judgements or evaluations in light of the first available thing that comes to mind (or is "available" to the mind). In the case of military hazard assessment, the ready availability of toxic gas models that predict the mean concentration, used in conjunction with the dosage-effect correlation that has been observed for some toxic materials, has led to the widespread use of the present hazard analysis methodology.

However, military hazard assessments that use only the mean concentration and a linear dosage ignore several important factors that may lead to serious errors: (1) the toxic effects produced by CBW agents are usually nonlinear with the important consequence that the toxic effects produced by these agents are dependent, on the level of concentration as well as the dosage (viz., for these agents a high peak concentration felt for a short duration, followed by a period of zero concentration, can cause a greater adverse effect than a low concentration felt for a longer time even though the dosage is the same in both exposures); (2) CBW agent concentrations in plumes dispersing in the atmosphere are inherently random variables because of their dependence on the fluctuations of a variety of meteorological (turbulence) and emission variables; and (3) the varying susceptibility in the exposed personnel to the toxic agent has not been properly accounted for. In view of these serious deficiencies in the current military CBW agent hazard assessment methodology, the purpose of this study is to develop a rational, yet simple, methodology for hazard assessment that overcomes the deficiencies cited. In particular, a model to account for the essentially statistical nature of atmospheric dispersion as it relates to the determination of an appropriate degree of injury function for exposure to CBW agents is developed.

Results

A rational, consistent, and simple methodology for the estimation of the degree of injury

or damage for exposed military personnel around a CBW agent release is developed. The hazard analysis procedure consists of a number of steps which can be summarized as follows. Firstly, the use of a nonlinear toxic load is advocated as a measure of the level of damage resulting from exposure to many harmful substances for which the interaction of concentration and exposure time is nonlinear. Evidence from a number of toxicological experiments supporting the use of the toxic load as an appropriate measure of the level of damage for a wide variety of toxic materials is summarized. Secondly, the importance of concentration fluctuations in determination of the toxic load is emphasized. Using an extensive new data set of instantaneous plume concentration measurements from the CONFLUX project, it is shown how various statistical characteristics can be determined and assigned to the fluctuating concentrations, and how the latter information can be utilized to provide a simple, practical method for estimating the ensemble-averaged (mean) toxic load in the presence of concentration fluctuations. Thirdly, the importance of identifying the relevant time scale of concentration fluctuations (e.g., a time scale relevant to the smoothing imposed by the human lungs) for the toxicological assessment is noted. Fourthly, the mathematical basis of probit relationships is reviewed, and their use in modeling the variability in individual toxic gas susceptibility is described. Fifthly, it is shown how by combining the ensemble-averaged toxic load and the probit method, it is possible to determine the fraction of an exposed population that would be affected by a given CBW agent event. The latter procedure simultaneously takes into account the intensity and frequency of concentration fluctuations in determination of the toxic load and the response of a population of varying susceptibility to this toxic load.

Significance of Results

With few exceptions, CBW agent dispersion models have been limited to the prediction of dosage or ensemble mean concentrations averaged over time scales of tens of minutes, hours, or more. While this scheme can be used successfully for predicting cumulative effects of nuclear radiation (e.g., radioactive isotopes), it is much less satisfactory for many CBW agents for which the interaction between concentration and exposure time is nonlinear. For example, the toxicity of many CBW agents may be increased by the existence of pockets of high concentration within the plume because the damage function (the so-called toxic load) is of the form C^nT, where n is an index (the so-called toxic load exponent) that is usually greater than I (typically in the range from 1 to 4), C is the concentration, and T is the exposure duration. In this case, the use of a time-averaged dosage could lead to a dangerous underestimation of the hazard with respect to both the severity of the toxic effect and the size or extent of the hazard zone.

In particular, actual measurements have shown that the fluctuation intensity (i.e., the ratio of the standard deviation of concentration to mean concentration) perceived at a time scale of about 1 s (e.g., a time scale comparable to the response of the human

lungs) varies in the range from about 0.5 to 10 depending on plume position. For toxic load exponents ranging from between 1 to 3.5 (viz., a range which covers that observed for a wide variety of toxic gases) and for the range of fluctuation intensities expected to be encountered in a dispersing plume, the ensemble-averaged toxic load ratio (viz., a ratio that measures the enhancement in the toxic response due to a fluctuating concentration over that provided by a steady, average concentration whose value is the mean of the fluctuating one) was found to vary from about 1 to greater than 1000. The enhancement in the toxic load predicted by the new model reinforces the importance of paying careful attention to plume meandering effects that can reduce mean-plume concentrations while increasing peak fluctuation levels at the plume fringes. These results provide compelling evidence for using probabilistic/statistical methods for military hazard assessment, rather than traditional deterministic methods.

Future Goals

With regard to the problem addressed in this paper, there is one significant area where further modeling work is required for practical application. The ensemble averaged (mean) toxic load was used for making quantitative estimates for biological response to toxic materials, largely because our current state of knowledge of quantities required for the prediction of the mean toxic load is relatively certain. However, a more complete scheme for hazard assessment would need to account for the inherent or natural variability in the nonlinear toxic load resulting from the concentration fluctuations. A difficult statistical problem that remains unsolved concerns the determination of the relationship between the statistical properties of the toxic load to those of the fluctuating concentration. In particular, an ambitious line of future research would be to develop a model for the probability distribution of the toxic load itself, and use this information in the toxicological assessment rather than the mean toxic load used here. However, it is important to note that the latter problem is extremely difficult because the probability distribution of the toxic load is not expressible in terms of the one-point probability distribution of the instantaneous concentration itself (for which reliable information is available at present), since it requires a knowledge about what is happening at many points in the plume simultaneously and, hence, of joint probability distributions of concentration (for which no Deformation is available at present).

William E. Lee, H. Gail Thompson, John G. Hall and Douglas E. Bader, "Rapid Detection of Chemical and Biological Agents by Immunoassay, Gene Probe Assay and Enzyme Inhibition Using a Silicon-Based Biosensor", **Suffield Report No. 636**, 1995.

Introduction

The CF have a well defined requirement to be able to operate in an area where CB agents might be used. A cornerstone of this requirement is the availability of field portable detection and identification technologies which will provide sufficient advanced warning to allow protective equipment to be used to avoid contamination and to identify the correct medical countermeasures to be employed on affected individuals. DRES is actively pursuing a program to develop novel biosensors which may be used to meet some of the requirement for CB detectors.

Biosensor technologies provide a rapid and sensitive means of detecting a wide variety of potential chemical and biological warfare (CB) agents. A sensor which measures the physiological effect of nerve agents (e.g., inhibition of the enzyme responsible for nervous control) provides a simple way to identify the class of chemical agents called nerve agents. For biological agents, a sensor which identifies the organism through antibody binding or genetic recognization is preferred. Advances in biosensor technology are allowing assay systems to be designed that can be automated and miniaturized without compromising the overall performance. This report describes a silicon-based sensor system, the Light Addressable Potentiometric (LAP) sensor, that can be used for detection and identification of both chemical and biological agents. The system, a light addressable potentiometric (LAP) sensor, can be used to measure antibody binding, genetic recognition and enzyme inhibition assays.

Results

Immunoassays have been developed for a number of biological agents derived from bacteria, virus and proteins. This work gives the results in terms of limits of detection in 0.1 mL samples for *Brucella melitensis* (2 ng), *bacillus globigii* (0.4 ng), Newcastle disease virus (0.2 ng) and staphylococcal enteroxin B (0.3 pg). A dual probe nucleic acid hybridization assay was shown to be suitable for the detection of DNA at a level of 0.3 fmoles (or 2 x 108 target molecules) per sample. The LAP sensor was shown to be effective for detection of aqueous samples of organophorus nerve agents. Soman and sarin could be detected in the range of 1-10 pg (per 50 μL sample). All of these detection limits are at or below requirements for field portable detection systems.

Significance of Results

This report demonstrates the utility of the LAP sensor to be able to provide detection and identification for a wide variety of threat agents in a routine manner. The apparatus

is compact (2 cu ft) and rugged, and as such, suitable for use in a field laboratory setting. In September 1995, it was successfully field tested as a component of an integrated chemical-biological mobile detection and identification laboratory.

Future Goals

The LAP sensor demonstrated very good performance in both laboratory and field trials. It is also being considered for inclusion in the advanced development model of a field portable chemical and biological agent detection system. However, at present it is a manual technology which requires substantial user expertise. Attempts will be made over the next few years to automate some of its functions and reduce the expertise needed to operate it effectively.

P.A. D'Agostino, J.R. Hancock and L.R. Provost, "Identification of Bioactive Peptides by High Resolution Liquid Chromatography - Electrospray Mass Spectrometry", **Suffield Report No. 637**, 1995.

Introduction

The Canadian Forces (CF) may be called on to perform peacekeeping or peacemaking operations in regions of the world where there is a significant threat of chemical/biological warfare agent use. To operate effectively in these theatres the CF must be able to identify the exact nature of the chemical/biological agent(s). Mass spectrometry (MS), is a powerful analytical technique for the identification of both known and unknown compounds and DRE Suffield is currently investigating this instrumental technique in fulfilment of CF agent identification requirements.

Results

A database of electrospray mass spectra (ESI-MS) for proteins which have biological activity was established during the development of MS techniques for identifying toxins. Mass spectra for a number of proteins which are simulants for mid-spectrum agents, including substance P (and related peptides), bradykinins, bombesins (and related peptides) and a Conus snail toxin, were acquired over a wide range of mass spectral conditions. Very accurate molecular weights were obtained for all of the materials analysed, and an excellent database of their mass spectral fingerprints was created. The peptide mass spectra contained in this database may now be used for identification of these materials in any samples provided.

Significance of Results

The CF may be deployed in regions of the world where there is a significant threat of chemical or biological warfare agent use. Identification of the agent is of importance since the results of such analyses would contribute to the development of strategic and political positions regarding future Canadian military operations and would facilitate the dissemination of technical advice to in-theatre field commanders and medical personnel.

Future Goals

The CB threat spectrum includes chemical and biological warfare agents, and toxins of biological origin in the "mid-spectrum" between these agents. The identification research effort has been focused on the detection and identification of these toxins of biological origin. Use of these warfare agents could easily go unconfirmed, as analytical methods have not been fully developed for their identification. DRE Suffield is now actively addressing this deficiency through the application and development of

MS methods for identification these agents.

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This document represents a collation of the publications for the Detection and Identification Section at the Defence Research Establishment Suffield for the calendar year of 1995 and January to May 1996. The publications have been divided into three types: (1) DRES publications; (2) Contractors' Reports, and; (3) External Publications.

The DRES Publications represent all of the in-house publications based on research and development in support of CF requirements. Executive summaries which emphasize the defence relevance of the work are included in this section, and full reports are available from DRES. The Contractors' Reports represent all of the final reports received for work conducted under contract to the Section. Although executive summaries are not included, the full reports are available from DRES. The External Publications section contains a bibliography of all of the articles published or submitted to scientific journals for publications. These articles are available from either DRES or any library which carries the appropriate journal.

The intent of this publication is to provide a brief review of the achievements of the Detection and Identification Section at DRES over the last 18 months in a format that is meant for both technical and non-technical audiences. If there are any questions about the program or any of the publications please contact the undersigned.

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